

I claim: —

1. A microcrystal comprising:
  - a) a derivatized protein selected from the group consisting of derivatized insulin, derivatized insulin analogs, and derivatized proinsulins;
  - b) a complexing compound;
  - c) a hexamer-stabilizing compound; and
  - d) a divalent metal cation.
2. The microcrystal of Claim 1, wherein the complexing compound is protamine which is present at about 0.15 mg to about 0.5 mg per 3.5 mg of derivatized protein.
3. The microcrystal of Claim 2, wherein the divalent metal cation is zinc, which is present at about 0.3 mole to about 0.7 mole per mole of derivatized protein.
4. The microcrystal of Claim 3, wherein the hexamer-stabilizing compound is a phenolic preservative selected from the group consisting of phenol, m-cresol, o-cresol, p-cresol, chlorocresol, methylparaben, and mixtures thereof and is present in sufficient proportions with respect to the derivatized protein to facilitate formation of the R6 hexamer conformation.
5. The microcrystal of Claim 4, wherein the derivatized protein is an acylated protein selected from the group consisting of acylated insulin and acylated insulin analogs.
6. The microcrystal of Claim 5, wherein the derivatized protein is a fatty acid-acylated insulin.
7. The microcrystal of Claim 6, wherein the derivatized protein is insulin that is acylated with a straight-chain, saturated fatty acid.

8. The microcrystal of Claim 7, wherein the derivatized protein is insulin that is mono-acylated at the LysB29-N $\epsilon$  amino group of insulin.

5 9. The microcrystal of Claim 8, wherein the derivatized protein is acylated with a fatty acid selected from the group consisting of n-hexanoic acid, n-heptanoic acid, n-octanoic acid, n-nonanoic acid, and n-decanoic acid.

10 10. The microcrystal of Claim 9, wherein the derivatized protein is selected from the group consisting of B29-N $\epsilon$ -hexanoyl-human insulin, B29-N $\epsilon$ -octanoyl-human insulin, and B29-N $\epsilon$ -decanoyl-human insulin.

15 11. The microcrystal of Claim 8, wherein the derivatized protein is acylated with a fatty acid selected from the group consisting of n-dodecanoic acid, n-tetradecanoic acid, and n-hexadecanoic acid.

20 12. The microcrystal of Claim 7, wherein the fatty acid-acylated insulin is a di-acylated insulin that is acylated at the LysB29-N $\epsilon$ -amino group and is also acylated at one N-terminal N $\alpha$ -amino group, and wherein the fatty acid is selected from the group consisting of n-hexanoic acid, n-heptanoic acid, n-octanoic acid, n-nonanoic acid, and n-decanoic acid.

25 13. The microcrystal of Claim 6, wherein the derivatized protein is insulin that is acylated with a branched-chain, saturated fatty acid.

14. The microcrystal of Claim 13, wherein the branched, saturated fatty acid has from three to ten carbon atoms in its longest branch.

30 15. The microcrystal of Claim 5, wherein the derivatized protein is a fatty acid-acylated insulin analog.

16. The microcrystal of Claim 15, wherein the derivatized protein is an insulin analog that is acylated with a straight-chain, saturated fatty acid.

17. The microcrystal of Claim 16, wherein the derivatized protein is mono-acylated at the N $\epsilon$ -amino group.

18. The microcrystal of Claim 17, wherein the derivatized protein is acylated with a fatty acid selected from the group consisting of n-hexanoic acid, n-heptanoic acid, n-octanoic acid, n-nonanoic acid, and n-decanoic acid.

19. The microcrystal of Claim 18, wherein the derivatized protein is selected from the group consisting of fatty acid-acylated animal insulins, fatty acid-acylated monomeric insulin analogs, fatty acid-acylated deletion analogs, and fatty acid-acylated pI-shifted insulin analogs.

20. The microcrystal of Claim 19, wherein the derivatized protein is fatty acid-acylated des(B30)-human insulin analog, fatty acid-acylated LysB28, ProB29-human insulin analog, or fatty acid-acylated AspB28-human insulin analog.

21. The microcrystal of Claim 20, wherein the derivatized protein is fatty acid-acylated des(B30)-human insulin analog.

22. The microcrystal of Claim 17, wherein the derivatized protein is acylated with a fatty acid selected from the group consisting of n-dodecanoic acid, n-tetradecanoic acid, and n-hexadecanoic acid.

23. The microcrystal of Claim 22, wherein the derivatized protein is selected from the group consisting of fatty acid-acylated animal insulins, fatty acid-acylated monomeric insulin analogs, fatty acid-acylated deletion analogs, and fatty acid-acylated pI-shifted insulin analogs.

24. The microcrystal of Claim 23, wherein the derivatized protein is fatty acid-acylated des(B30)-human insulin analog, fatty acid-acylated LysB28, ProB29-human insulin analog, or fatty acid-acylated AspB28-human insulin analog.

26. The microcrystal of Claim 25, wherein the  
5 derivatized protein is B29-Nε-myristoyl-des(B30)-human  
insulin analog.

10            28. The microcrystal of Claim 15, wherein the derivatized protein is an insulin analog that is acylated with a branched-chain, saturated fatty acid.

29. The microcrystal of Claim 28, wherein the branched chain, saturated fatty acid has from three to ten carbon atoms in its longest branch.

30. The microcrystal of Claim 1, wherein the microcrystal has rod-like morphology.

31. The microcrystal of Claim 1, wherein the microcrystal has irregular morphology.

20 32. A suspension formulation comprising an insoluble phase and a solution phase, wherein the insoluble phase is comprised of the microcrystal of Claim 1, and the solution phase is comprised of water.

25 33. A suspension formulation comprising an insoluble phase and a solution phase, wherein the insoluble phase is comprised of the microcrystal of Claim 2 and the solution phase is comprised of water.

34. The suspension formulation of Claim 33, wherein the solution phase is further comprised of a phenolic preservative at a concentration of about 0.5 mg per mL to about 6 mg per mL of solution, a pharmaceutically acceptable buffer, and an isotonicity agent.

35. The suspension formulation of Claim 34, wherein the solution phase is further comprised of insulin, an insulin analog, an acylated insulin, or an acylated insulin analog.

5 36. The suspension formulation of Claim 35, wherein the solution phase is comprised of insulin.

37. The suspension formulation of Claim 35, wherein the solution phase is comprised of an insulin analog.

10 38. The suspension formulation of Claim 37, wherein the insulin analog is a monomeric insulin analog.

39. The suspension formulation of Claim 38, wherein the insulin analog is LysB28, ProB29-human insulin analog.

15 40. The suspension formulation of Claim 32, wherein the solution phase is further comprised of zinc and protamine, wherein the ratio of zinc to derivatized protein in the suspension formulation is from about 5 to about 7 mole of zinc atoms per mole of derivatized protein, and the  
20 ratio of protamine to derivatized protein in the suspension formulation is from about 0.25 mg to about 0.5 mg per mg of derivatized protein.

41. A process for preparing the microcrystal of Claim 1 comprising:

- 25 a) dissolving a derivatized protein, a hexamer-stabilizing compound, and a divalent metal cation in an aqueous solvent having a pH that will permit the formation of hexamers of the derivatized protein, and  
30 b) adding a complexing compound.

42. A process for preparing the microcrystal of Claim 1 comprising:

- a) dissolving a derivatized protein, a hexamer-stabilizing compound, and a divalent metal cation in an aqueous solvent having a pH that will not permit the formation of hexamers of the derivatized protein, and
- b) adjusting the pH to between about 6.8 and about 7.8; and
- c) adding a complexing compound.

43. A method of treating diabetes comprising administering the formulation of Claim 32 to a patient in need thereof in a quantity sufficient to regulate blood glucose levels in the patient.

44. An amorphous precipitate comprising:

- a) a derivatized protein selected from the group consisting of derivatized insulin, derivatized insulin analogs, and derivatized proinsulins;
- b) a complexing compound;
- c) a hexamer-stabilizing compound; and
- d) a divalent metal cation.

45. The amorphous precipitate of Claim 44, wherein the complexing compound is protamine which is present at about 0.15 mg to about 0.5 mg per 3.5 mg of derivatized protein.

46. The amorphous precipitate of Claim 45, wherein the divalent metal cation is zinc, which is present at about 0.3 mole to about 0.7 mole per mole of derivatized protein.

47. The amorphous precipitate of Claim 46, wherein the hexamer-stabilizing compound is a phenolic preservative selected from the group consisting of phenol, m-cresol, o-cresol, p-cresol, chlorocr sol, methylparaben, and mixtures thereof and is present in sufficient proportions with

respect to the derivatized protein to facilitate formation of the R6 hexamer conformation.

48. The amorphous precipitate of Claim 47, wherein the derivatized protein is an acylated protein selected from the group consisting of acylated insulin and acylated insulin analogs.

49. The amorphous precipitate of Claim 48, wherein the derivatized protein is a fatty acid-acylated insulin.

50. The amorphous precipitate of Claim 49, wherein the derivatized protein is insulin that is acylated with a straight-chain, saturated fatty acid.

51. The amorphous precipitate of Claim 50, wherein the derivatized protein is insulin that is mono-acylated at the LysB29-N $\epsilon$  amino group of insulin.

52. The amorphous precipitate of Claim 51, wherein the derivatized protein is acylated with a fatty acid selected from the group consisting of n-hexanoic acid, n-heptanoic acid, n-octanoic acid, n-nonanoic acid, and n-decanoic acid.

53. The amorphous precipitate of Claim 52, wherein the derivatized protein is selected from the group consisting of B29-N $\epsilon$ -hexanoyl-human insulin, B29-N $\epsilon$ -octanoyl-human insulin, and B29-N $\epsilon$ -decanoyl-human insulin.

54. The amorphous precipitate of Claim 51, wherein the derivatized protein is acylated with a fatty acid selected from the group consisting of n-dodecanoic acid, n-tetradecanoic acid, and n-hexadecanoic acid.

55. The amorphous precipitate of Claim 50, wherein the fatty acid-acylated insulin is a di-acylated insulin that is acylated at the LysB29-N $\epsilon$ -amino group and is also acylated at one N-terminal N $\alpha$ -amino group, and wherein the fatty acid is selected from the group consisting of n-hexanoic acid, n-heptanoic acid, n-octanoic acid, n-nonanoic acid, and n-decanoic acid.

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56. The amorphous precipitate of Claim 49, wher in the derivatized protein is insulin that is acylated with a branched-chain, saturated fatty acid.

57. The amorphous precipitate of Claim 56,  
5 wherein the branched, saturated fatty acid has from three to ten carbon atoms in its longest branch.

58. The amorphous precipitate of Claim 48, wherein the derivatized protein is a fatty acid-acylated insulin analog.

10 59. The amorphous precipitate of Claim 58, wherein the derivatized protein is an insulin analog that is acylated with a straight-chain, saturated fatty acid.

15 60. The amorphous precipitate of Claim 59, wherein the derivatized protein is mono-acylated at the Nε-amino group.

20 61. The amorphous precipitate of Claim 60, wherein the derivatized protein is acylated with a fatty acid selected from the group consisting of n-hexanoic acid, n-heptanoic acid, n-octanoic acid, n-nonanoic acid, and n-decanoic acid.

25 62. The amorphous precipitate of Claim 61, wherein the derivatized protein is selected from the group consisting of fatty acid-acylated animal insulins, fatty acid-acylated monomeric insulin analogs, fatty acid-acylated deletion analogs, and fatty acid-acylated pI-shifted insulin analogs.

30 63. The amorphous precipitate of Claim 62, wherein the derivatized protein is fatty acid-acylated des(B30)-human insulin analog, fatty acid-acylated LysB28, ProB29-human insulin analog, or fatty acid-acylated AspB28-human insulin analog.

64. The amorphous precipitate of Claim 63, wherein th derivatized protein is fatty acid-acylated des(B30)-human insulin analog.



65. The amorphous precipitate of Claim 60, wherein the derivatized protein is acylated with a fatty acid selected from the group consisting of n-dodecanoic acid, n-tetradecanoic acid, and n-hexadecanoic acid.

5 66. The amorphous precipitate of Claim 65, wherein the derivatized protein is selected from the group consisting of fatty acid-acylated animal insulins, fatty acid-acylated monomeric insulin analogs, fatty acid-acylated deletion analogs, and fatty acid-acylated pI-shifted insulin analogs.

10 67. The amorphous precipitate of Claim 66, wherein the derivatized protein is fatty acid-acylated des(B30)-human insulin analog, fatty acid-acylated LysB28,ProB29-human insulin analog, or fatty acid-acylated AspB28-human insulin analog.

15 68. The amorphous precipitate of Claim 67, wherein the derivatized protein is fatty acid-acylated des(B30)-human insulin analog.

20 69. The amorphous precipitate of Claim 68, wherein the derivatized protein is B29-Nε-myristoyl-des(B30)-human insulin analog.

70. The amorphous precipitate of Claim 69, wherein the derivatized protein is B28-Nε-myristoyl-LysB28,ProB29-human insulin analog.

25 71. The amorphous precipitate of Claim 58, wherein the derivatized protein is an insulin analog that is acylated with a branched-chain, saturated fatty acid.

30 72. The amorphous precipitate of Claim 71, wherein the branched chain, saturated fatty acid has from three to ten carbon atoms in its longest branch.

73. A suspension formulation comprising an insoluble phase and a solution phase, wherein the insoluble phase is comprised of the amorphous precipitate of Claim 44, and the solution phase is comprised of water.

74. A suspension formulation comprising an insoluble phase and a solution phase, wherein the insoluble phase is comprised of the amorphous precipitate of Claim 45 and the solution phase is comprised of water.

5 75. The suspension formulation of Claim 41, wherein the solution phase is further comprised of a phenolic preservative at a concentration of about 0.5 mg per mL to about 6 mg per mL of solution, a pharmaceutically acceptable buffer, and an isotonicity agent.

10 76. The suspension formulation of Claim 75, wherein the solution phase is further comprised of insulin, an insulin analog, an acylated insulin, or an acylated insulin analog.

15 77. The suspension formulation of Claim 76, wherein the solution phase is comprised of insulin.

78. The suspension formulation of Claim 76, wherein the solution phase is comprised of an insulin analog.

20 79. The suspension formulation of Claim 78, wherein the insulin analog is a monomeric insulin analog.

80. The suspension formulation of Claim 79, wherein the insulin analog is LysB28, ProB29-human insulin analog.

25 81. The suspension formulation of Claim 73, wherein the solution phase is further comprised of zinc and protamine, wherein the ratio of zinc to derivatized protein in the suspension formulation is from about 5 to about 7 mole of zinc atoms per mole of derivatized protein, and the ratio of protamine to derivatized protein in the suspension  
30 formulation is from about 0.25 mg to about 0.5 mg per mg of derivatized protein.

82. A process for preparing the amorphous precipitate of Claim 45 comprising:

a) dissolving a derivatized protein, a hexamer-stabilizing compound, and a divalent metal cation in an aqueous solvent having a pH that will permit the formation of hexamers of the derivatized protein, and

b) adding a complexing compound.

83. A process for preparing the amorphous precipitate of Claim 45 comprising:

a) dissolving a derivatized protein, a hexamer-stabilizing compound, and a divalent metal cation in an aqueous solvent having a pH that will not permit the formation of hexamers of the derivatized protein, and

b) adjusting the pH to between about 6.8 and about 7.8; and

c) adding a complexing compound.

84. A method of treating diabetes comprising administering the formulation of Claim 73 to a patient in need thereof in a quantity sufficient to regulate blood glucose levels in the patient.

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